**Human Papilloma Virus Attributable Head and Neck Cancer in the Sudan Assessed by p16\textsuperscript{INK4A} Immunostaining**

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**Abstract**

**Background:** The aim of this study was to screen for human papillomavirus (HPV) infections in head and neck squamous cell carcinomas (HNSCCs) using P16 immunostaining. **Materials and Methods:** A retrospective study was performed on 150 samples from patients diagnosed with HNSCCs. HPV status was determined using p16\textsuperscript{INK4A}. **Results:** 31 of the 150 (20.7%) HNSCCs were HPV positive. **Conclusions:** A large proportion of HNSCCs in Sudan are associated with HPV infection. The fact that the prevalence of HPV is high among Sudanese patients with head and neck cancers (HNC) has obvious implications for vaccine therapy.

**Keywords:** Head and neck cancer - human papillomavirus - p16\textsuperscript{INK4A} - Sudan

**Introduction**

Head and neck (HNC) is the eighth most common cause of cancer death worldwide (Ferlay, 2010). Its incidence varies widely among different regions. In North America and the Europe, HNC accounts for 3-4% of all cancer diagnoses. On the other hand, in Southeast Asia and Africa, HNC accounts for approximately 8-10% of all cancers (Santarelli et al., 2009). It is well known that there is a strong association between gene, environment and cancer. Several factors are involved in oral carcinogenesis, such as age, gender, ethnicity, lifestyle, genetic background, status of health and exposure to one or more oncogenic factors (Llewellyn et al., 2004). In several epidemiologic studies, tobacco smoking and alcohol consumption have been well documented as major risk factors for HNC (Castellsague et al., 2004).

It is now evident that a significant proportion of HNSCCs, are caused by HPV (Chung and Gillison, 2009). High-risk HPV subtype 16 accounts for more than 85% of all HPV-positive (HPV+) tumors in HNSCC (Dayyani et al., 2010). Patients with HPV-positive HNSCC had a lower risk of dying, and a lower risk of recurrence than HPV-negative HNSCC patients (Fakhry et al., 2008).

Knowledge of HPV and EGFR status can have implications for treatment options and prognosis in HNSCC. The investigation of oncogenic gene expression in HPV-related OSCC and the study of its potential value as a predictor of neoplastic progression and clinical outcome could allow to characterize a possible evaluative morphological profile of HNC. As a rule, the expression of HPV markers and surrogate markers of HPV infection can be easily evaluated by IHC. Different antigens should be used including virus related (capsidic antigens, E5-E6-E7 proteins), and virus induced and/or altered host proteins (p16\textsuperscript{INK4A}, pRb, Cyclin proteins, p-53) (Pannone et al., 2011).

Several authors have emphasized that a hallmark of the presence of HPV in cancer could be found in p16 nuclear or cytoplasmic overexpression, so that p16 could be considered a useful surrogate marker for HPV (Gillespie et al., 2009; Goon et al., 2009).

Therefore, the aim of this study was to determine the prevalence HPV infections in head and neck squamous cell carcinomas (HNSCCs) amongst Sudanese patients.

**Materials and Methods**

A total of 150 patients, 90 males and 60 females (male/female ratio, 1.5:1), aged between 12 and 85 years with mean age of 54 years, were diagnosed as having HNCs, were investigated for the presence of HPV different genotypes using p16 immunohistochemistry. The diagnosis was based on clinical examination and histological features of the biopsy. HNCs diagnosis was verified base on Royal College of Pathologists criteria (Royal College of Pathologists, 2005). The HNCs including 144/150 (96%) squamous cell carcinoms (SCCS) and 6/150 (4%) adenocarcinoma.

The sample included full coverage of patients with HNC lesions referred to our hospital within Two-year time. Ethical consent was obtained from ethical committee of the Faculty Research Board and Hospital.

P16 immunohistochemistry (IHC) was performed on

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formalin-fixed paraffin embedded (FFPE) tissue sections using kits from (Beijing Aide Lai Biotechnology Co., Ltd.). After antigen retrieval, sections were incubated with mouse monoclonal anti-p16, and then EnVision_ System HRP anti-mouse, followed by diaminobenzidine chromogen and counterstaining with hematoxylin. Cervical cancer sections known to be HPV-positive were used as a positive control, and omission of primary antibody was used as a negative control. All p16 IHC slides were semiquantitatively scored by two investigators for intensity of staining in the cell nucleus and cytoplasm. Intensity was scored as 0 (none), 1(weak), 2 (moderate), or 3 (strong), with 0 or 1 scores defined as negative and 2 or 3 defined as positive. P16 scoring was performed without knowledge of HPV status.

Results

In the present study we investigated 150 head and neck carcinomas, obtained from different anatomical sites including oral, larynx, pharynx, esophagus and others, constituting, 51, 19, 17, 53 and 10 respectively, as shown in Figure 1.

Of 150 tested HNCs tested for the presence of HPV, P16 was found positive in 31/150 (20.7%). All of the positive samples were from SCCs. Of the 31 positive cases, 18/31 (58%) were found among males and 13/31 (42%) were detected among females. High frequencies of HPV positive were found in oral site cancer followed by esophagus, larynx, pharynx and others, representing, 11/31 (35.4%), 7/31 (22.6%), 5/31 (16%), 4/31 (13%) and 4/31 (13%), in this order. Notably, when computing these values from entire group, the proportion of percentages changes in different sites, as indicated in Figure 2.

In regard to age, the highest frequency of infection occurred in age range 31-40 year followed by age groups 51-60 and 61-70 representing 10/31 (32%), 6/31 (19%) and 6/31 (19%), respectively. Nevertheless, when computing the proportions of percentages for each group entirely, the values changes as seen in Figure 3.

According to occupation, most HPV-positive cases were among housewives and employees constituting 10/31 (32%) for each, followed by labors and students representing 9/31 (29%) and 2/31 (6%), respectively. Furthermore, the proportions of percentages for each occupation entirely were described in Figure 4.

Discussion

The association of head and neck cancers with clinically momentous morbidity and disfiguration makes early detection of the diseases and biomarkers to identify individuals at high risk of great importance. Although, HPV infection is emerging as an important risk factor for several HNCs, but it is a powerful prognostic biomarker in these cancers.

Previous studies have shown that patients with HPV positive tumors actually benefit from a better overall disease-specific survival than patients with HPV-negative tumors (Deng et al., 2012). In this study we used p16 immunostaining to screen tissue samples obtained from patients with HNCs to indicate the pre-existence of HPV.
infection. The current study has indicated a significant association between HPV infection and HNSCCs in Sudan, and to the best of our knowledge this is the first report in this context from Sudan. In a study investigating the prevalence of human papillomaviruses (HPV), in 155 OSCCs from eight different countries from different ethnic groups, continents and with different socioeconomic backgrounds; the highest prevalence of HPV was seen in Sudan (65%) (Jalouli et al., 2012). Although, our findings showed lower percentages compared to these reports, which might be due to differences in methodologies, but results support some investigations in the oral cancer from Sudan. A study from Sudan evaluated the possible role of high risk HPV 16 and 18 in oral squamous cell carcinomas (OSCC), 40 SCCs and 15 benign lesions, HPV DNA was detected in 15% of cases (six out of 40 cases), and none of controls (n=15), P<0.0001 (Ahmed and Eltoom, 2010). Moreover, there are some studies investigated the relationship between oral cancer and HPV infection. Of these studies, a study found that HPV was in only 2 Sudanese cases, both of which harbored both type 6 and type 11: both these cases demonstrated mild epithelial dysplasia (Ibrahim et al., 1998).

In the present study, HPV infections were more frequent identified in the tumor tissues from oral (40%) followed by larynx and pharynx. These findings were in agreement with the study that reported, HPV prevalence was 35.6% in oropharyngeal cancers, 23.5% in oral cancers and 24.0% in laryngeal cancers (Goon, et al., 2009). Nevertheless, many studies have suggested that tobacco use and alcohol consumption increase the risk of head and neck cancer (Coelho, 2012; Zhou et al., 2012). Whether HPV is an independent risk factor of tobacco and alcohol, the other two major causes of tumors at these sites, has not been well clarified (Herrero, et al., 2003; Applebaum et al., 2007; Gillison et al., 2008; Smith et al., 2010a). It is known that those who are infected with HPV have significantly better survival (Klussmann et al., 2003; Smith et al., 2010b), thus it would be important to clarify the role of these risk factors.

According to occupation most HPV infections were found among employees followed by housewives. However, a link has been demonstrated between social class and HPV-related cancers. Data indicates that cervical cancer incidence is considerably higher among women of working age in manual than in non-manual classes (Rushton et al., 2010; Parkin, 2011). The impact of occupational exposures, together with the occupational circumstances and industrial areas where exposures to carcinogenic agents occurred in the past, on population cancer morbidity and mortality; this can be compared with the impact of other causes of cancer (Parkin, 2011).

One of the limitations of this study is that the results were not confirmed with molecular techniques, such as ISH or PCR.

The current study provides support for the contributing role of HPV infection in etiology of HNSCCs in Sudan. Further studies with more specific techniques are recommended to measure the real burden of HPV in etiology of HNCs, this in addition to provide information about different genotypes.

References


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